IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appellants:	C. UEMATSU, et al.
Application. No.	10/759,179
Filed:	JANUARY 20, 2004
For:	METHOD FOR EXPRESSED GENE ANALYSIS AND PROBE KIT FOR EXPRESSED GENE ANALYSIS
Group AU:	1637
Examiner:	Stephanie Kane Mummert
Confirm. No.:	7828

APPEAL BRIEF

Mail Stop: APPEAL BRIEF
Honorable Commissioner of
Patent and Trademarks
P.O. Box 1450
Alexandria, VA 22313-1450

September 25, 2007

Sir:

This Appeal Brief is in connection with an Appeal from the decision in the Office Action mailed January 24, 2007, in the above-identified application, finally rejecting claims 1-9 and 14. No claim is allowed.

REAL PARTY IN INTEREST

The real party in interest in connection with the subject matter claimed in the above-identified application is Hitachi, Ltd., the Assignment having been recorded on January 20, 2004, at Reel 014906/Frame 0904.

RELATED APPEALS AND INTERFERENCES

Upon information and belief, there are no known prior or pending appeals, interferences, or judicial proceedings which may be related to, directly affect or be directly affected by or have a bearing on the Board's decision in this pending appeal.

STATUS OF CLAIMS

Claims 1-9 and 14 have been finally rejected in the Final Office Action mailed January 24, 2007 and are the subject matter of this appeal. Claims 10-13 stand withdrawn from consideration, in light of the oral election reported in the Office Action mailed December 29, 2005, in the above-identified application.

There are no allowed claims in the above-identified application.

Thus, the claims on appeal are claims 1-9 and 14, the only claims being considered on the merits in the above-identified application.

STATUS OF AMENDMENTS

No amendment was filed after Final rejection, in the above-identified application. A Request for Reconsideration After Final Rejection was submitted April 24, 2007, and was considered in the Advisory Action mailed May 24, 2007.

SUMMARY OF CLAIMED SUBJECT MATTER

Of the claims on appeal, the only independent claim is claim 1. Claim 1 defines a method for expressed gene analysis. The method as claimed in clam 1 comprises subjecting a gene to be analyzed to nucleic acid amplification, using a forward primer specifically hybridizing to the gene to be analyzed, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, and comprising a second base sequence closer to the 5' end of the primer than the first base sequence, and a probe including a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase, and ribonuclease H and/or exonuclease. The method of claim 1 also includes digesting the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification (note that this ribonuclease H or exonuclease is at an end of the probe); and detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification. Independent claim 1 goes on to recite, in a final "wherein" clause, that the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence.

As for the processing recited in claim 1, this is described most generally in the paragraph bridging pages 3 and 4 of Appellants' specification.

Note, in particular, the first full paragraph on page 4 of Appellants' specification, beginning at line 6 thereof. Note especially the disclosure in this paragraph that although the third sequence varies depending on the target gene sequence, the first sequence can be freely designed independent of the target gene sequence, and that the second sequence is a constant sequence independent of the target gene sequence (e.g., T7 promoter sequence).

Note also the description in the first paragraph on page 6 of Appellants' specification, that in the method of the present invention, nucleic acid amplification after reverse transcription is carried out at a substantially single temperature using a pair of primers, this term "substantially single temperature" referring to the temperature at which enzymes such as reverse transcriptase, ribonuclease H, RNA polymerase and exonuclease simultaneously have enzyme activity, this single temperature being, specifically, about 35ºC-55ºC (note appealed claims 5 and 6, claim 5 being dependent on claim 1 and claim 6 being dependent on claim 5). This paragraph goes on to describe that at this temperature, reverse transcription, synthesis of double-stranded cDNA, transcription, and probe digestion simultaneously proceed, but that double-stranded DNA comprising several hundred or more nucleotides such as genomic DNA are not generally denatured in this temperature range; and that, accordingly, genomic DNA other than the target gene is not amplified in the process for expressed gene analysis of the present invention, so that through use of specified temperatures, the target gene can be specially detected without generating any reaction by-product.

Note also the description in the second paragraph on page 6 of Appellants' specification that the probe for detection can be freely designed regardless of the target gene sequence, and thus it can be universally used regardless of the type of the target gene.

Note also Fig. 1 of Appellants' original disclosure, and the description in connection therewith on page 8, line 8 to page 10, line 6, of Appellants' specification, showing, inter alia, the nucleic acid amplification, with the primer 11 for introduction being constituted by the sequence portion 12 hybridizing to the target RNA, the sequence portion 13 located closer to the 5' end than the sequence portion 12 and consisting of a sequence identical to the probe for detection, and the sequence portion 14 comprising a T7 promoter sequence located closer to the 5' end than the sequence portion 13. This shows the design of the primer for introduction, as well as design of the primer and universal probe, and synthesis of the gene to be analyzed.

Note also the paragraph bridging pages 10 and 11 of Appellants' specification, together with Fig. 3 of Appellants' disclosure, describing the mechanism of the probe 15 for emitting fluorescence.

Of the dependent claims, claims 5 and 6, reciting that the nucleic acid amplification is conducted at a substantially single temperature, in particular, a temperature between 37°C and 55°C, have been previously discussed.

Claim 2, dependent on claim 1, recites that the gene to be analyzed is cDNA comprising the first and second base sequences introduced therein by the introduction with subjecting the mRNA of the target gene to reverse transcription using the primer for introduction recited in claim 1. In connection with this method of claim 2, note, for example, the paragraph bridging pages 4 and 5 of Appellants' specification.

Claims 3 and 4, each dependent on claim 1, each further defines the nucleic acid amplification, as being conducted by sequentially repeating steps 1) - 3) set forth in these two claims. Steps 1) and 2) of these two claims are the same, reciting transcription of the gene to be analyzed into RNA with the aid of RNA polymerase, and reverse transcription of the RNA using the forward primer and the reverse

transcriptase or ribonuclease H to synthesis single-stranded cDNA. Claims 3 and 4 differ in step 3), step 3) of claim 3 reciting synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase, and step 3) of claim 4 reciting synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and the reverse transcriptase (rather than DNA polymerase as in claim 3). In connection with claims 3 and 4, note page 5, lines 7-20, of Appellants' specification.

Claim 7, dependent on claim 1, recites that the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence. Note, for example, Figs. 2(a) and (b) of Appellants' original disclosure, together with the description in connection therewith on page 10, lines 11-24 of Appellants' specification.

Claim 8, dependent on claim 1, defines the additional feature of the present invention wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes. In connection therewith, note Fig. 4 and the description corresponding thereto from page 14, line 4, through page 18, line 8, of Appellants' specification. As can be appreciated, by this additional feature of the present invention, several (e.g., two or more) target genes can be simultaneously detected in a single reaction vessel, using two or more types of probes. Claim 9, dependent on claim 8, recites that the melting temperatures (Tm values) of the at least two probes are substantially the same; as is clear from the first full paragraph on page 15 of Appellants' specification, the melting temperatures (TM values) of the two or more types of probes can be substantially the same, and therefore the probes can simultaneously react in a same reaction tube.

Claim 14, dependent on claim 1, recites that the probe is a DNA/RNA hybrid strand, as described in the paragraph bridging pages 10 and 11 of Appellants'

specification. As described in this paragraph bridging pages 10 and 11 of Appellants' specification, and in the first full paragraph on page 11 thereof, the sequence of the probe 15 (note Fig. 1) can be designed independent of the sequence of target RNA; and when assaying another target RNA, redesign of the sequence portion 12 of the reverse transcription primer 11 and the sequence of the forward primer 10 is sufficient, and the probe 15 can be used as a universal probe.

GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

A first ground of rejection to be reviewed on appeal is the rejection of claims 1-9 under 35 U.S.C. §103(a) as being unpatentable over the teachings of Wenz, et al., U.S. Patent Application Publication No. 2003/0190646, in view of Ovin, et al., U.S. Patent No. 6,110,681, Livak, et al., U.S. Patent No. 5,538,848, and further in view of the article by Eun, et al., "Simultaneous Quantitation of Two Orchid Viruses, by the TaqMan ® Real-Time RT-PCR", in Journal of Virological Methods 87 (2000), pages 151-160.

A second ground of rejection presented for review in the rejection of claims 1-7 under 35 U.S.C. §103(a) as unpatentable over Wenz, et al., in view of the article of Leone, et al., "Molecular Beacon Probes Combined with Amplification by NASBA Enable Homogeneous, Real-Time detection of RNA", in Nucleic Acids Research, 1998, Vol. 26, No. 9, pages 2150-2155 (Leone '98) as evidenced by the article by Leone, et al., "Direct Detection of Potato Leafroll Virus in Potato Tubers by immunocapture and the Isothermal Nucleic Acid Amplification Method NASB", in Journal of Virological Methods 66 (1997), pages 19-27 (Leone '97), Bass, U.S. Patent Application Publication No. 2001/0039014, and further in view of the article by Mackay, et al., "Real-time PCR in Virology", in Nucleic Acids Research, 2002, Vol. 30, No. 6, pages 1292-1305.

A third ground of rejection to be reviewed on appeal is the rejection of claim 14 under 35 U.S.C. §103(a) as unpatentable over Wenz, et al., in view of Ovyn, et al., and further in view of the article by Rizzo, et al., "Chimeric RNA-DNA Molecular Beacon Assay for Ribonuclease H Activity", in Molecular and Cellular Probes (2002) 16, pages 227-283.

ARGUMENTS

It is respectfully submitted that the combined teachings of Wenz, et al., in view of Ovyn, et al., and Livak, et al., and further in view of the article by Eun, et al., would have neither taught nor would have suggested the subject matter of claims 1-9. It is further respectfully submitted that the combined teachings of Wenz,et al., in view of Leone '98 as evidenced by Leone '97 and Bass, et al., and further in view of the article by Mackay, et al., would have neither taught nor would have suggested the subject matter of claims 1-7. It is respectfully submitted that the combined teachings of Wenz, et al., in view of Ovyn, et al., and further in view of the article by Rizzo, et al., would have neither taught nor would have suggested the subject matter of claim 14.

REJECTION OF CLAIMS 1-9 UNDER 35 U.S.C. §103(a) AS UNPATENTABLE OVER WENZ, ET AL., IN VIEW OF OVYN, ET AL., AND LIVAK, ET AL., AND FURTHER IN VIEW OF THE ARTICLE OF EUN, ET AL.

CLAIM 1

It is respectfully submitted that the combined teachings of Wenz, et al., in view of Ovyn, et al., and Livak, et al., and further in view of Eun, et al., would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, including a step of subjecting the gene to be analyzed to nucleic acid amplification, using, inter alia, (a) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence and including a second base sequence closer to the 5' end of the primer than the first base sequence, and (b) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at ends thereof respectively with a fluorophore and with a quencher, reverse transcriptase,

RNA polymerase and ribonuclease H and /or exonuclease, together with steps of digesting the probe and detecting fluorescence, and wherein the gene to analyzed is prepared by the introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase into the target gene, so that the second base sequence is bound to a position closer to a 5' end of the gene to an analyzed than the first base sequence. See claim 1.

It is respectfully submitted that none of the references, even taking the teachings thereof in combination, disclose or would have suggested subjecting the gene to be analyzed to amplification using the primer for introduction including the first base sequence (a target nonspecific sequence) at a location between a third base sequence (a target-specific sequence) at the 3' end and a second base sequence (e.g., T7 promoter sequence) at the 5' end, and by introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase into the target gene, in the manner specified in the present claims. Through such introduction and use of the recited nonspecific first base sequence and the probe comprising, inter alia, a base sequence identical or complementary to the first base sequence, an advantage is achieved that the probe used in the present invention does not have to be designed for each use in accordance with the base sequence of the target gene and can be universally used regardless of the target gene. The probe used can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. See, e.g., pages 28 and 29 of Appellants' specification.

It is respectfully submitted that with the probe described in Wenz, et al., the "AS-SP" portion cannot hybridize with a target, and the probe cannot extend from the

"AS-SP" portion, because the "T-SP" portion is located at the 5' end of the probe and the "P-SP" portion is located at the 3' end of the probe. Especially in view thereof, it is respectfully submitted that the probe disclosed in Wenz, et al., including the "T-SP" portion, the "AS-SP" portion and the "P-SP" portion, with the "AS-SP" portion between the "T-SP" and the "P-SP" portions, is functionally different from the primer having the first through third sequences, of the present invention.

Moreover, it is emphasized that the present claims recite a method, including the positive processing step of subjecting a gene to be analyzed to nucleic acid amplification using the specified forward primer, the primer for introduction and the probe, discussed previously. While Appellants disagree with any characterization by the Examiner of Wenz, et al., as disclosing a probe/primer functionally equal to the primer/probe of the present invention, even if this characterization were correct this does not provide a basis for concluding that the reference discloses the subjecting step of claim 1, noting additional features of the "subjecting" step as in the wherein clause of claim 1.

CLAIM 2

Moreover, it is respectfully submitted that the combined teachings of Wenz, et al., Ovyn, et al., Livak, et al., and the article by Eun, et al., as applied by the Examiner, would have neither taught nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having the feature of claim 2 wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction.

When mRNA of a certain target gene is to be detected, a primer for introduction that includes the stated three sequence portions is first used as a reverse transcription primer to synthesize first strand cDNA from target RNA (mRNA), this cDNA functioning as a gene to be analyzed prepared by introducing the first and second base sequences to the target gene; and, subsequently, the obtained gene to be analyzed is used as a template to synthesize second strand cDNA. Thus, a gene to be analyzed having the promoter sequence of RNA polymerize is synthesized. Note, the paragraph bridging pages 4 and 5 of Appellants' specification.

CLAIMS 3 AND 4

In addition, it is respectfully submitted that the teachings of the references set forth in the heading to this section would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and additionally having the feature of conducting nucleic acid amplification by sequentially repeating the steps (1) - (3), including, in connection with claim 3, synthesis of the gene to be analyzed from single-stranded cDNA using the primer for introduction and DNA polymerase (3); or, in connection with claim 4, synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and the reverse transcriptase.

According to this aspect of the present invention, the synthesized gene to be analyzed is used as a template in transcription in step 1), and steps 1) - 3) are sequentially repeated, such that amplification proceeds. Note, for example, the first and second full paragraphs on page 5 of Appellants' specification.

CLAIMS 5 AND 6

It is respectfully submitted that the combined teachings of Wenz, et al., Ovyn, et al., Livak, et al., and the article by Eun, et al., as applied by the Examiner, would have neither taught nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1, and additionally wherein the nucleic acid amplification is conducted at a substantially single temperature as in claim 5, more specifically, wherein this single temperature is between 37°C and 55°C as in claim 6.

Due to use of such single temperature, genomic DNA other than the target gene is not amplified in the process for expressed gene analysis of the present invention; and, more specifically, by simply conducting incubation at a certain temperature, the target gene can be specially detected without generating any reaction by-products. Note the first paragraph on page 6 of Appellants' specification.

CLAIM 7

It is respectfully submitted that the combined teachings of Wenz, et al., Ovyn, et al., Livak, et al., and the article by Eun, et al., as applied by the Examiner, would have neither taught nor would have suggested the method for expressed gene analysis as in claim 7, having features as discussed previously in connection with claim 1, and additionally wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence.

This specific design, for the specific T7 RNA polymerase, would not have been suggested by the combined teachings of the application references.

CLAIM 8

It is respectfully submitted that the combined teachings of Wenz, et al., Ovyn, et al., Livak, et al., and the article by Eun, et al., as applied by the Examiner, would have neither taught nor would have suggested the method for expressed gene analysis as in claim 8, having features as discussed previously in connection with claim 1, and additionally wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes. See claim 8.

According to this aspect of the present invention, described on pages 16-18 of Appellants' specification and as seen in Fig. 5, several target genes can be simultaneously detected. Moreover, accuracy of detection can be enhanced in the diagnosis of infections, since several items for one diagnostic subject can be evaluated in one operation. See in particular the first full paragraph on page 18 of Appellants' specification.

In addition, by performing the simultaneous detection in a single reaction vessel, different, e.g., viruses can be subjected to typing in a single reaction vessel, which enables evaluation of several items at a time and at a single location, with direct comparison of types and fluorescence intensities of fluorophores, whereby typing accuracy can be improved. Note, for example, pages 18-21 of Appellents' specification, and in particular the first two paragraphs on page 21 thereof.

CLAIM 9

In addition, it is respectfully submitted that the teachings of Wenz, et al., Ovyn, et al., Livak, et al., and the article by Eun, et al., as applied by the Examiner, would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as set forth in each of claims

1 and 8, and additionally wherein the melting temperatures (TM values) of the probes are substantially the same. See claim 9.

By having such TM values being substantially the same, reaction properties of each probe can be controlled at the same levels, thereby conducting accurate expression analysis. Note the fourth full paragraph on page 6 of Appellants' specification.

REJECTION OF CLAIMS 1-7 UNDER 35 U.S.C. §103 OVER THE COMBINED TEACHINGS OF WENZ, ET AL., IN VIEW OF LEONE ('98) AS EVIDENCED BY LEONE ('97), BASS, ET AL., AND FURTHER IN VIEW OF THE ARTICLE BY MACKAY, ET AL.

CLAIM 1

It is respectfully submitted that the combined teachings of Wenz, et al., in view of Leone ('98) as evidenced by Leone ('97), Bass, et al., the article by Mackay, et al. would have neither taught nor would have suggested such a method for expressed gene analysis as in the present claims, including a step of subjecting the gene to be analyzed to nucleic acid amplification, using, inter alia, (a) a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence and including a second base sequence closer to the 5' end of the primer than the first base sequence, and (b) a probe comprising a base sequence identical or complementary to the first base sequence and labeled at ends thereof respectively with a fluorophore and with a quencher, reverse transcriptase, RNA polymerase and ribonuclease H and/or exonuclease, together with steps of digesting the probe and detecting fluorescence, and wherein the gene to analyzed is prepared by the introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a

promoter sequence of RNA polymerase, into the target gene, so that the second base sequence is bound to a position closer to a 5' end of the gene to an analyzed than the first base sequence. See claim 1.

It is respectfully submitted that none of the references, even taking the teachings thereof in combination, disclose or would have suggested subjecting the gene to be analyzed to amplification using the primer for introduction including the first base sequence (a target nonspecific sequence) at a location between a third base sequence (a target-specific sequence) at the 3' end and a second base sequence (e.g., T7 promoter sequence) at the 5' end, and by introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase into the target gene, in the manner specified in the present claims. Through such introduction and use of the recited nonspecific first base sequence and the probe comprising, inter alia, a base sequence identical or complementary to the first base sequence, an advantage is achieved that the probe used in the present invention does not have to be designed for each use in accordance with the base sequence of the target gene and can be universally used regardless of the target gene. The probe used can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. See, e.g., pages 28 and 29 of Appellants' specification.

It is respectfully submitted that with the probe described in Wenz, et al., the "AS-SP" portion cannot hybridize with a target, and the probe cannot extend from the "AS-SP" portion, because the "T-SP" portion is located at the 5' end of the probe and the "P-SP" portion is located at the 3' end of the probe. Especially in view thereof, it is respectfully submitted that the probe disclosed in Wenz, et al., including the "T-SP" portion, the "AS-SP" portion and the "P-SP" portion, with the "AS-SP" portion

between the "T-SP" and the "P-SP" portions, is functionally different from the primer having the first through third sequences, of the present invention.

Moreover, it is emphasized that the present claims recite a method, including the positive processing step of subjecting a gene to be analyzed to nucleic acid amplification using the specified forward primer, the primer for introduction and the probe, discussed previously. While Appellants disagree with any characterization by the Examiner of Wenz, et al., as disclosing a probe/primer functionally equal to the primer/probe of the present invention, even if this characterization were correct this does not provide a basis for concluding that the reference discloses the subjecting step of claim 1, noting additional features of the "subjecting" step as in the wherein clause of claim 1.

CLAIM 2

Moreover, it is respectfully submitted that the combined teachings of Wenz, et al., Leone ('98) as evidenced by Leone ('97), Bass, et al. and Mackay, et al., as applied by the Examiner, would have neither taught nor would have suggested such method for expressed gene analysis as in the present claims, having features as in claim 1 as discussed previously, and, additionally, having the feature of claim 2 wherein a gene to be analyzed is cDNA including the first and second base sequences introduced therein by the introduction with subjecting mRNA of the target gene to reverse transcription using the primer for introduction. See claim 2.

When mRNA of a certain target gene is to be detected, a primer for introduction that includes the stated three sequence portions is first used as a reverse transcription primer to synthesize first strand cDNA from target RNA (mRNA), this cDNA functioning as a gene to be analyzed prepared by introducing the first and second base sequences to the target gene; and, subsequently, the

obtained gene to be analyzed is used as a template to synthesize second strand cDNA. Thus, a gene to be analyzed having the promoter sequence of RNA polymerize is synthesized. Note, the paragraph bridging pages 4 and 5 of Appellants' specification.

CLAIMS 3 AND 4

In addition, it is respectfully submitted that the teachings of the references set forth in the heading to this section would have neither disclosed nor would have suggested such method for expressed gene analysis as in the present claims, having features as discussed previously in connection with claim 1, and additionally having the feature of conducting nucleic acid amplification by sequentially repeating the steps (1) - (3), including, in connection with claim 3, synthesis of the gene to be analyzed from single-stranded cDNA using the primer for introduction and DNA polymerase (3); or, in connection with claim 4, synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and the reverse transcriptase.

According to this aspect of the present invention, the synthesized gene to be analyzed issued as a template in transcription in step 1), and steps 1) - 3) are sequentially repeated, such that amplification proceeds. Note, for example, the first and second full paragraphs on page 5 of Appellants' specification.

CLAIMS 5 AND 6

It is respectfully submitted that the combined teachings of the references set forth in the heading to this section, as applied by the Examiner, would have neither taught nor would have suggested such method for expressed gene analysis as in the present claims, wherein the nucleic acid amplification is conducted at a substantially

single temperature as in claim 5, more specifically, wherein this single temperature is between 37ºC and 55ºC as in claim 6.

Due to the use of such single temperature, genomic DNA other than the target gene is not amplified in the process for expressed gene analysis of the present invention; and, more specifically, by simply conducting incubation at a certain temperature, the target gene can be specially detected without generating any reaction bi-product. Note, the first paragraph on page 6 of Applicants' specification.

CLAIM 7

It is respectfully submitted that the combined teachings of the references set froth in the heading to this section, as applied by the Examiner, would have neither taught nor would have suggested the method for expressed gene analysis as in claim 7, having features as discussed previously in connection with claim 1, and additionally wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence.

REJECTION OF CLAIM 14 UNDER 35 U.S.C. §103 AS UNPATENTABLE OVER WENZ, ET AL., IN VIEW OF OVYN, ET AL., AND FURTHER IN VIEW OF THE ARTICLE BY RIZZO, ET AL.

It is respectfully submitted that the teachings of the references as applied in rejecting claim 14 would have neither taught nor would have suggested such method for expressed gene analysis as in claim 14, having features as in claim 1, and wherein the probe is a DNA/RNA hybrid strand.

By use of the primer for introduction as in all of the present claims, which includes the first, second and third base sequences located relative to the 5' end of the primer, with the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of

RNA polymerase, the first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, together with the probe comprising a base sequence identical or complementary to the first base sequence, a universal probe for expressed gene analysis which does not have to be designed for each use in accordance with the base sequence of the target gene is achieved. The universal probe according to the present invention can amplify and detect any type of target gene under substantially the same conditions, and analysis thereof can be simply conducted. Note, for example, the second paragraph on page 6 of Appellants' specification; see also the paragraph bridging pages 28 and 29 thereof.

Wenz, et al. discloses a technique for detection of nucleic acid sequences using coupled legation and amplification reactions. The most general disclosure of the technique described in Wenz, et al. is set forth in paragraph [0007] on page 1 of this patent document. Note also paragraphs [0009] and [0010] on page 1; the Examiner has specifically referred to paragraphs [0028] - [0030] on page 3 of this patent document, and Figs. 1-3 of this patent document.

Wenz, et al. uses a probe including a T-SP portion (a target-specific sequence), a "P-SP" portion (a target nonspecific sequence), and an "AS-SP" portion between the "T-SP" portion and the "P-SP" portion. It is respectfully submitted that the "AS-SP" portion cannot hybridize with a target, and the probe cannot extend from the "AS-SP" portion, because the "T-SP" portion is located at the 5' end of the probe and the "P-SP" portion is located at the 3' end of the probe. It is respectfully submitted that Fig. 1 of Wenz, et al. clearly shows this fact, that is, the probe of Wenz, et al. cannot be used as a primer. It is respectfully submitted that Fig. 3 (cont.), on sheet 4 of 7 of the Wenz, et al. publication, shows that an oligonucleotide including the "P-SP", the "AS-SP" and "P-SP" portions in Wenz, et al. is used as a

template, in contrast to a primer. As can be seen in the foregoing, it is respectfully submitted that the three portions described in Wenz, et al. are functionally different from the first, second and third base sequences of the present invention. That is, it is respectfully submitted that the probe described in Wenz, et al. is completely different from the primer for introduction in the present invention, in function as well as in construction.

On the second page of the Advisory Action mailed May 24, 2007, the Examiner points to the specification of Wenz, et al., at paragraph [0029] on page 3 thereof, as describing that each probe includes a portion that is complementary to or substantially complementary to the target (the "target specific portion" T-SP) and a portion that is complementary to or has the same sequence as a primer (the "primer specific portion" P-SP), and states that at least one probe in each probe set further comprises an addressable support-specific portion (AS-SP) that is located between the T-SP and the P-SP; and contends that, therefore, in view of this teaching, it would have been obvious to one of ordinary skill to incorporate the same components of the probe where the AS-SP portion is incorporated at the 5' end of the primer and with the T-SP portion at the 3' end, the opposite of what is depicted in Fig. 1. From this, the Examiner asserts that when the components of the probe/primer are modified in this way, the probe could function as a primer instead of as a ligation probe as depicted in Fig. 1. This assertion by the Examiner is respectfully traversed. Even were the probe/primer to be modified as asserted by the Examiner, it is respectfully submitted that the present invention cannot be achieved by such modified structure. The present invention is characterized in that "the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene

so that the second base sequence is bound to positions closer to the 5' end of the gene to be analyzed than the first base sequence", and is also characterized by the use of "a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with [inter alia] a quencher". Even with such modification as asserted by the Examiner, it is respectfully submitted that Wenz, et al., either alone or in combination with the teachings of the other references as applied by the Examiner, would have neither taught nor would have suggested such features of the present invention as characterized in the foregoing. It is respectfully submitted that the effect of the present invention is that the probe for detection can be designed and used regardless of the target gene sequence, and any type of target gene can be amplified and detected under substantially the same conditions and analysis thereof can be simply conducted. It is respectfully submitted that this effect cannot be achieved by the structure modified as asserted by the Examiner.

In addition, it is noted that the Examiner indicates that the probe "could" function as a primer, and does not even allege that the references suggest such functioning. The Examiner has not alleged, and it is respectfully submitted cannot properly allege, that Wenz, et al., either alone or in combination with the teachings of the other references as applied, would have taught the method as in the present claims, including, inter alia, preparation of the gene to be analyzed and use of the probe as in the present claims.

Contentions by the Examiner concerning Wenz, et al., in the paragraph bridging pages 19 and 20 of the Office Action mailed January 24, 2007, are noted. However, contrary to the contentions by the Examiner, it is respectfully submitted that the addressable-specific portion of the probe in Wenz, et al. does not serve the same functional role as the first base sequence as in the present claims; and, in

particular, it is respectfully submitted that the addressable-specific portion of the primer in Wenz, et al. cannot hybridize with a target, and the probe cannot extend from the AS-SP portion. Particularly in view of this error by the Examiner in interpreting the teachings of Wenz, et al., it is respectfully submitted that the conclusion by the Examiner of obviousness of the presently claimed subject matter is in error.

Moreover, it is respectfully emphasized that, as a further difference (deficiency) between the teachings of Wenz, et al. and the presently claimed subject matter, Wenz, et al. would have neither taught nor would have suggested such method as in the present claims, including, inter alia, use of the primer for introduction including the first base sequence closer to the 5' end of the probe than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a specified base sequence, as part of the processing as in the present claims, and advantages thereof as discussed previously.

It is respectfully submitted that the additional teachings of the secondary references as applied by the Examiner in Items 2-4 on pages 2-18 of the Final Office Action mailed January 24, 2007, would not have rectified the deficiencies of Wenz, et al., such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Ovyn, et al. discloses oligonucleotides that can be used as primers to amplify a region of the 16S rRNA of *Mycoplasma pneumoniae*. Note column 3, lines 39-45 of this patent. Note especially column 7, lines 5-28, of this patent, describing a method for the detection of the specified microorganism. Note also column 3, lines 39-46; column 5, lines 46-67; and column 6, lines 12-16 and 43-61.

It is respectfully submitted that Ovyn, et al. would have neither taught nor would have suggested, alone or in combination with the teachings of the other

applied references, the presently claimed method, including, inter alia, use of the primer for introduction having the first base sequence as in the present claims, together with the second and third base sequences.

It is respectfully submitted that the upstream and downstream primers in Ovyn, et al. only comprise, respectively, a sequence substantially complementary to the target sequence and a sequence substantially homologous to the target sequence. It is respectfully submitted that this patent would have neither taught nor would have suggested, inter alia, a first base sequence as in the present claims.

Moreover, it is respectfully submitted that the probe described in Ovyn, et al. does not include "a base sequence identical or complementary to the first base sequence". It is respectfully submitted that Ovyn, et al. would have neither taught nor would have suggested such probe including such base sequence identical or complementary to the first base sequence, as in the present claims, and advantages thereof as discussed previously.

Livak, et al. discloses methods of monitoring the process of nucleic acid amplification reactions, especially polymerase chain reactions. Note, in general, column 3, lines 29-47, for the broadest description of this method. See also column 3, lines 48-55.

Eun, et al. discloses simultaneous quantitation of two orchid viruses carried out using the TaqMan® real-time RT-PCR. As for the primer design for the method disclosed in Eun, et al., note Table 1 and the description in Item 2.2 on page 153 of this article.

Even assuming, arguendo, that the teachings of Livak, et al. and of Eun, et al. were properly combinable with the teachings of Wenz, et al., and Ovyn, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including, inter alia, the base sequences, including

in particular, the first base sequence used together with the second and third base sequence in the primer for introduction, and/or the probe including a base sequence identical or complementary to the first base sequence, and labeled at one end with a fluorophore and at another end with, inter alia, a quencher, or preparation of the gene to be analyzed as in the "wherein" clause at the end of claim 1, and advantages achieved thereby; and/or other features of the present invention as discussed in the foregoing, and advantages thereof.

It is respectfully submitted that the teachings of the references as applied by the Examiner in Items 3 and 4 on pages 9-18 of the Final Office Action mailed January 24, 2007, would have neither taught nor would have suggested the presently claimed subject matter, including features thereof as discussed previously.

Wenz, et al. has been previously discussed.

Leone '98 discloses employment of molecular beacon probes in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. This article describes the coupling of RNA amplification by NASBA with amplicon detection by molecular beacon technology to produce a homogenous RNA assay, called AmpliDet RNA. Note the first full paragraph in the left-hand column on page 2151 of this article. See also the discussions under the headings "Selection of amplification primers and probe", "Synthesis of the molecular beacons", "NASBA" and "Post-NASBA analysis", on page 2151 of this article.

Leone '97 reports on the development and optimization of the NASBA technology for the direct detection of PLRV (potato Leafroll virus) virions in microfuge tubes and to a post-NASBA analysis amplification products by acridine orange-stained agarose gels. Note the sole full paragraph in the right-hand column on page 20 of Leone '97.

Taking the teachings of Leone '98 even as evidenced by the disclosure of Leone '97, and even in light of the teachings of other references as applied by the Examiner, discussed infra, it is respectfully submitted that the combined teachings of the references would have neither taught nor would have suggested the presently claimed subject matter, including, inter alia, use of the primer for introduction having the first base sequence as in the present claims, and other features of the presently claimed method, and advantages thereof as discussed previously.

Bass, et al. discloses automated devices and systems for performing nucleic acid recombination, mutation, shuffling and other diversity generating reactions in vitro. As applied by the Examiner, this publication discloses that as an alternative to TaqMan® is the use of molecular beacons to assess library quality. Note paragraph [0329] on page 36.

The article by MacKay, et al., reports on detection of polymerase chain reaction products during real-time. As applied by the Examiner, note, for example, page 1297, right-hand column, of this article.

Even assuming, arguendo, that the teachings of Wenz, et al., Leone '98 (even as evidenced by Leone '97), Bass, et al. and Mackay, et al. were properly combinable, it is respectfully submitted that such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including use of the primer for introduction having the first base sequence, as in the present claims, and other features of the present invention as discussed previously, including the probe with a base sequence identical or complementary to the first base sequence, and preparation of the gene to be analyzed, and advantages thereof.

In connection with the rejection of claim 14 as set forth in Item 4 on pages 15-18 of the Office Action mailed January 24, 2007, the teachings of Wenz, et al. and Ovyn, et al., have been previously discussed.

The article by Rizzo, et al. discloses preparation of RNA/DNA chimeric molecular beacons, which contain a single-stranded RNA/DNA chimeric oligonucleotide labeled with a 5'-fluorescein as fluorophore and a 3'-DABCYL as quencher, referring to Fig. 1 on page 279 of this article. This article discloses that the fluorophore of the probe is held in proximity to the quencher by the stem-loop structure; and that when the RNA sequence of the RNA:DNA hybrid stem is cleaved, the fluorescence of the fluorophore is manifested. Note the second full paragraph in the left-hand column on page 278 of this article. Note also the paragraph on pages 279 and 280; and the Conclusions set forth in the left-hand column on page 282.

Even assuming, arguendo, that the teachings of Rizzo, et al., were properly combinable with the teachings of Wenz, et al., and of Ovyn, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed subject matter, including use of the primer for introduction comprising the first base sequence between the second and third sequences, the first base sequence closer to the 5' end of the primer than the third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence as specified in claim 1, or the probe comprising, inter alia, a base sequence identical or complementary to the first base sequence and labeled at the ends as set forth in claim 1, or wherein the gene to be analyzed is prepared by introduction of the first base sequence which is nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase.

CONCLUSION

It is respectfully submitted that the Examiner errs in concluding that the combined teachings of Wenz, et al., Ovyn, et al., and the article by Eun, et al., would have taught the subject matter of claims 1-9 on appeal. It is also respectfully submitted that the Examiner errs in concluding that the combined teachings of Wenz, et al., Leone '98 as evidenced by Leone '97, Bass, et al., and the article by MacKay, et al., would have taught the subject matter claimed in claims 1-7 on appeal. It is also respectfully submitted that the Examiner errs in concluding that the combined teachings of Wenz, et al., Ovyn, et al., and the article by Rizzo, et al., would have taught the subject matter of claim 14 on appeal. Accordingly, it is respectfully submitted that the rejections by the Examiner of claims 1-9 and 14 as set forth in the Final Office Action mailed January 24, 2007, are in error, and the Honorable Board is respectfully requested to correct these errors by reversing the rejections of claims 1-9 and 14, in due course.

The Appeal Brief fee in the amount of $500.00 is attached hereto.

Please charge any shortage in fees due in connection with the filing of this paper to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case No. 1021.43414X00), and please credit any excess fees to such Deposit Account.

Respectfully submitted,

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CLAIMS APPENDIX

1. A method for expressed gene analysis comprising:

subjecting a gene to be analyzed to nucleic acid amplification using a forward primer specifically hybridizing to the gene to be analyzed, a primer for introduction comprising a first base sequence closer to the 5' end of the primer than a third base sequence comprising a sequence specifically hybridizing to a target gene, the target gene having a base sequence, and comprising a second base sequence closer to the 5' end of the primer than the first base sequence, a probe comprising a base sequence identical or complementary to the first base sequence and labeled at one end with a fluorophore and at another end with a quencher, reverse transcriptase, RNA polymerase, and ribonuclease H and/or exonuclease;

digesting the probe bound to the first base sequence by the ribonuclease H or exonuclease at the time of the nucleic acid amplification; and

detecting fluorescence emitted by the released fluorophore, thereby assaying the amount of the product of the nucleic acid amplification,

wherein the gene to be analyzed is prepared by the introduction of the first base sequence being nonspecific to the base sequence of the target gene and the second base sequence comprising a promoter sequence of RNA polymerase, into the target gene so that the second base sequence is bound to a position closer to a 5' end of the gene to be analyzed than the first base sequence.

2. The method for expressed gene analysis according to claim 1, wherein the gene to be analyzed is cDNA comprising the first base sequence and the second base sequence introduced therein by the introduction with subjecting the mRNA of the target gene to reverse transcription using the primer for introduction which comprises the first base sequence, which is closer to the 5' end of the primer than

the third base sequence comprising a sequence that specifically hybridizes to the target gene and the second base sequence, which is closer to the 5' end of the primer than the first base sequence.

3. The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

1) transcription of the gene to be analyzed into RNA with the aid of RNA polymerase;

2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and

3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and DNA polymerase.

4. The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted by sequentially repeating the following steps 1) to 3):

1) transcription of the gene to be analyzed into RNA with the aid of RNA polymerase;

2) reverse transcription of the RNA using the forward primer and the reverse transcriptase or ribonuclease H to synthesize single-stranded cDNA; and

3) synthesis of the gene to be analyzed from the single-stranded cDNA using the primer for introduction and the reverse transcriptase.

5. The method for expressed gene analysis according to claim 1, wherein the nucleic acid amplification is conducted at a substantially single temperature.

6. The method for expressed gene analysis according to claim 5, wherein the single temperature is between 37ºC and 55ºC.

7. The method for expressed gene analysis according to claim 1, wherein the RNA polymerase is T7 RNA polymerase and the second base sequence comprises the T7 promoter sequence.

8. The method for expressed gene analysis according to claim 1, wherein two or more target genes are simultaneously detected in a single reaction vessel using two or more types of probes.

9. The method for expressed gene analysis according to claim 8, wherein the melting temperatures (Tm values) of the two or more types of probes are substantially the same.

14. The method for expressed gene analysis according to claim 1, wherein the probe is a DNA/RNA hybrid strand.

EVIDENCE APPENDIX

None.

RELATED PROCEEDINGS APPENDIX

None.